Analysis of the Arabidopsis CDC2a Promoter (MOLECULAR BIOLOGY AND INFORMATION-Molecular Biology)

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Analysis of the *Arabidopsis CDC2a* Promoter

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The eukaryotic cell division cycle is tightly controlled by a class of protein kinases. *Arabidopsis CDC2a* has been considered to encode one of those protein kinases because it is expressed in proliferating tissues and can complement defects in the *cdc2* gene of *Schizosaccharomyces pombe*. The promoter of *CDC2a* was investigated as a first step in exploring the regulation of plant cell proliferation. We found that its transcription is started at the position 677-base-pairs upstream from the *CDC2a* initiation codon. To localize the *cis*-element for proliferating-cell-specific expression, histochemical analysis was done with *β*-glucuronidase fusion genes containing various upstream regions of *CDC2a*. Results from the experiment indicated that a region downstream from the transcription start site is required for the proliferating-cell-specific expression of *CDC2a* and that an upstream region contains a *cis*-element directing transcription during trichome development.

Keywords: Cell cycle/ Transcription start site/ Transgenic plant/ GUS fusion/ Trichome

Proliferation of eukaryotic cells is tightly controlled according to a common cell cycle program. At various points of the cell cycle, specific protein kinase activities are required. Genetic studies of cell division in fission yeast *Schizosaccharomyces pombe* have identified the product of the *cdc2* gene ($p34^{cdc2}$) as a key component of those kinase activities (for reviews, see 1 and 2). $p34^{cdc2}$ is a catalytic subunit of such kinases and its activity is regulated by association with cyclin and post-translational modifications throughout the cell cycle (for reviews see 2-4).

Many genes encoding protein kinases similar to $p34^{cdc2}$ have been cloned from higher plants so far. In *Arabidopsis*, two *cdc2*-related genes, *CDC2a* and *CDC2b*, have been identified (5, 6). Yeast complementation analysis have demonstrated that *CDC2a*, but not *CDC2b*, encodes a functional homolog of $p34^{cdc2}$ (5,6). During plant development, accumulation of *CDC2a* mRNA is correlated with cell proliferation and with increased competence for cell division in certain tissues (7,8). From these facts, transcriptional regulation of *CDC2a* is thought to be closely linked to the regulation under which plant cells are destined to proliferate. In this work, the promoter of *CDC2a* was investigated as a first step in exploring the regulation of plant cell proliferation.

We first determined the transcription start site of *CDC2a*. Total RNA was prepared from seedlings of wild type *Arabidopsis thaliana* (Columbia ecotype) and subjected to primer extension and S1-nuclease mapping. An intense signal band at the position 677-base-pairs (bp) upstream from the

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Scope of research

Attempts have been made to elucidate structure-function relationships of genetic materials and various gene products. The major subjects are mechanisms involved in signal transduction and regulation of gene expression responsive to environmental stimuli, development of plant leaves and flowers, and plant-microbe interaction. As of December 1996, study is being concentrated on (1) roles of homeodomain proteins and MADS box proteins in developmental processes and transcriptional control in higher plants and (2) contribution of protein phosphorylation and dephosphorylation toward cell cycle control and signal transduction in plants and plant pathogens.

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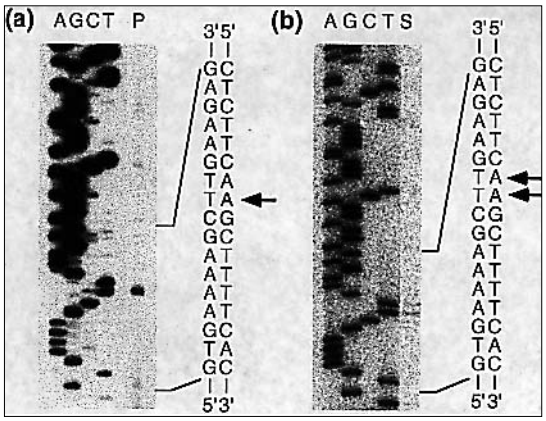

Figure 1. Primer-extension and S1-mapping analyses of the CDC2a promoter. The products of the reverse transcriptase reaction (lane P) (a) and the S1-nuclease reaction (lane S) (b) were electrophoresed together with reference sequence ladders (lanes A, G, C, and T). All the labeled products have the same 5' end so that the position of the signals can be referred easily. The sequence of the both strands in the relevant region are shown on each right side and the signal positions are indicated by arrows.

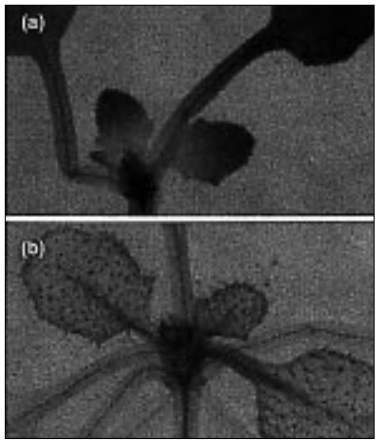

Figure 2. Histochemical analysis of the *CDC2a* promoter. Transgenic *Arabidopsis* plants 2-weeks-old after germination were examined histochemically: (a) transgenic plant carrying 35S-GUS; (b) transgenic plant carrying -591/+4-GUS, respectively.

CDC2a initiation codon and signal bands around the same position were observed in the primer-extension and the S1-mapping analysis, respectively (Figure 1). We concluded that the transcription of the *CDC2a* gene is started at the position 677-bp upstream from the initiation codon.

Activity of the *CDC2a* promoter in proliferating cells has been demonstrated in both histochemical analysis with a translational β-glucuronidase (GUS) fusion gene (8) and *in situ* hybridization analysis (7). In order to localize the *cis*-element required for the proliferating-cell-specific activity, we constructed three GUS fusion genes and introduced them into transgenic *Arabidopsis*. The GUS-coding sequence is preceded by the fragment between the positions 1301-bp upstream and 4-bp downstream from the transcription start site in the fusion gene designated as -1301/+4-GUS, and by the fragment between the positions 591-bp upstream and 4-bp downstream in that designated as -591/+4-GUS. The other designated as -986/+680-GUS is a translational fusion gene in which the fragment between the position 986-bp upstream from the transcription start site and the *CDC2a* initiation codon is fused to the GUS-coding sequence in an in-frame manner.

Several independent lines of transgenic plants for each GUS fusion gene were examined histochemically at the stage of juvenile plants 2-weeks-old after germination. Transgenic plants carrying the GUS-coding sequence preceded by the cauliflower mosaic virus 35S promoter (9) was used as a positive control of GUS staining (Figure 2a). Expression of -986/+680-GUS was detected in apical shoot and root meristems (data not shown) as reported before (7 and 8). On the other hand, -591/+4-GUS conferred no GUS activity to meristematic cells (Figure 2b). Instead, developing trichome cells showed strong GUS activity in plants carrying the fusion gene (Figure 2b). As trichomes maturated, the GUS activity gradually decreased and finally disappeared (data not shown). The same pattern of GUS expression was observed with -1301/+4-GUS (data not shown).

We concluded from these results that a region downstream from the transcription start site is required for the proliferating-cell-specific activity of the *CDC2a* promoter. Our results indicate another interesting fact that the 595-bp region located just upstream from the transcription start site contains a *cis*-element acting during trichome development. In *Arabidopsis*, a trichome is a unicellular organ existing on the leaf surface and its development consists of unique processes including endoreplication, extraordinary cell enlargement, and complicated cell morphogenesis (for review, see 10). The 595-bp region directs transcription during one of these unique processes. From a view point of protein function, endoreplication is a highly probable process because CDC2a function might be needed for DNA replication repeated without mitosis. CDC2a might act in outgrowth of trichome cells as CDK5 plays a critical role in nurite outgrowth during neuronal differentiation (11). In order to examine these possibility, it will be required to identify the responsible *cis*-element and the developmental process in which the *cis*-element is involved.

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